Teaching Points

They will work in pairs – but each student should end up with their own 50 ml centrifuge tube of spun down bacteria to put in the freezer.

Encourage them to fill in their lab notebooks as they do the lab.

It is **due** when they are done with the lab before they leave for Spring Break.

You will be teaching them sterile technique for the first time – so be strict so that they get in the practice of doing it correctly from the beginning

Be SUPER careful of the gas burners! – they don’t appreciate how dangerous they can be.

They should never leave the burner on if they leave the area.

Get the E. coli competent cells out LAST. They have to work with these quickly.

THEY SHOULD SPLIT 1 tube

The heat shock must be EXACTLY 42OC for 45 seconds.

For the ‘FUN’ plate – they can take a Kim Wipe or a cotton swab and swipe it on anything they think is ‘dirty’ (e.g. the lab floor, the sink, etc.) Then see what grows.

Be sure to explain to them what each things is for:

Competent cells (made to easily uptake DNA)

Plasmid – circular DNA with their gene of interest

Heat shock – opens up pores on membrane to uptake DNA (or makes more ‘fluid’)

LB– nutrient and energy rich media

Agar – makes it gel like (solid)

Ampicillin – plasmid has beta-lactamase gene which is the enzyme that breaks down Ampicillin

Log growth – the ‘happy’ phase for cells to be in when they produce the most protein

Lysozyme – enzyme that breaks down bacterial cell walls (peptidoglycan)

You don’t need to mention IPTG induction since we don’t use it in this expression. – but you might mention it if you feel comfortable talking about it because they are gonna use it in the fall.

Be sure they know that they don’t spin down their sample until it is PURPLE – which means they may have to wait til the next day (Day 4)

**Things they can do in advance to speed up later steps:**

* PreLab calculations: especially amount of AMP to add
* Find their LB and AMP and PBS stock that they made in Lab 1
* Figure out what they are going to put on their labels
  + Pre-label their 14ml Transformation tubes
  + Pre-label their 14 ml Small culture tubes
  + Make 1x PBS

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